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# Cultivation of *Neochloris oleoabundans* in bubble column photobioreactor with or without localized deoxygenation

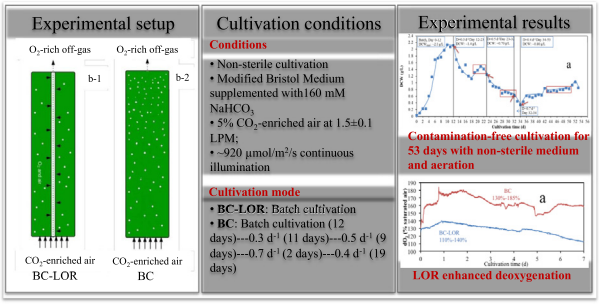
Licheng Peng [a], Zisheng Zhang [a], Peiyao Cheng [a], Zhaohui Wang [b], Christopher Q. Lan [a,*]

[a] Department of Chemical and Biological Engineering, University of Ottawa, Ottawa, ON K1N 6N5, Canada
[b] Department of Chemical Engineering, Nanjing Tech University, Nanjing, China

## HIGHLIGHTS

- Localized oxygen remover (LOR) enhanced deoxygenation efficiency.
- Low $dO_2$ beneficial to lipid accumulation in cells.
- Pigment cell content varied with time in batch cultivation but stable in continuous culture.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This study evaluated long-term non-sterile cultivation of freshwater green alga *Neochloris oleoabundans* in a 15-liter bubble column photobioreactor (BCPBR) and the effects of a membrane-based localized oxygen remover (LOR) on deoxygenation, cell growth, and lipid production of *N. oleoabundans*. Batch and continuous cultivations were carried out under non-sterile conditions for 53 days with no detectable protozoa or other biological contaminants, indicating successful long-term contamination-free cultivation. The results show that the BCPBR equipped with LOR (BCPBR-LOR) has enhanced deoxygenation efficiency and were able to maintain dissolved oxygen at a level of around 120% air saturation, which was 32% lower than that of the conventional BCPBR, which had no LOR. While similar biomass concentration and productivity were obtained in both systems, significantly higher lipid cell content and lipid productivity of microalgae were obtained in the latter, which was attributed to the low $dO_2$ in culture due to enhanced deoxygenation efficiency of BCPBR-LOR.



## 1. Introduction

Alga-derived biofuels are a promising substitute for fossil fuels in the future. However, the intensive energy demands required for algal cultivation such as sterilization, mixing, aeration, and the resulting high costs of cultivation have been the major barriers that prevent large-scale commercial production of microalgal biofuels (Borowitzka, 2013). Among these, the control of biological contamination and de-oxygenation are two of the major contributors. Due to the low biomass concentration of microalgal culture as a consequence of light limitation, large volumes of cultures have to be processed to achieve moderate biomass production. As a result, sterilization of large volumes of media becomes essential for sterile cultivation of microalgae, which is very energy intensive and costly (Muller-Feuga et al., 2012). Needless to say, the closed cultivation system required for sterile cultivation is much more expensive than the open systems for non-sterile cultivation. Furthermore, autotrophic cultivation of microalgae relies on capture of solar

* Corresponding author.
*E-mail address:* Christopher.Lan@uottawa.ca (C.Q. Lan).

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energy as energy source for microalgal cell growth, which demands the cultivation system to have a large surface-to-volume ratio for efficient light harvest and distribution (Wang et al., 2012), making the maintenance of sterility of a microalgal cultivation system inherently difficult and costly.

Freshwater microalga *Neochloris oleoabundans* has been established for high lipid content and ideal feedstock of biodiesel production (Li et al., 2008b) and capable to convert inexpensive sugars such as lactose to biopolymers (Wu et al., 2011). It has also demonstrated great potentials for $CO_2$ mitigation and wastewater treatment (Wang and Lan, 2011a). More recent studies have shown that adding appropriate amount of $NaHCO_3$ in media (Peng et al., 2015a) and controlling cultures pH at optimal range (Peng et al., 2015b) would result in selective inhibition of protozoa and therefore enable its safe cultivation in open non-sterile systems. Nevertheless, these studies were carried out in 3-liter bench-top bioreactors or smaller vessels such as cultivation bottles or flasks in batch mode for relative short period, typically 12 days or less. In this study, we demonstrated with a 15-liter bubble column photobioreactor (BCPBR) that long-term continuous contamination-free cultivation of freshwater microalga *N. oleoabundans* under non-sterile conditions is feasible.

On another front, cell-evolved oxygen may accumulate in cultures and pose severe threats to microalgae (Peng et al., 2013). These threats range from photochemical inhibition to photosynthetic apparatus damage (Ugwu et al., 2007), destruction of chlorophylls and other cellular compounds (Santabarbara et al., 2002) and, in the worst case scenario, collapse of culture at high oxygen concentrations (Ugwu et al., 2007). Thus, oxygen accumulation is one of the major constraining factors for microalgal cultures, particularly for large-scale systems (Sánchez Mirón et al., 1999). A number of different de-oxygenation approaches, which include paddle wheel (Jiménez et al., 2003), static mixer (Ugwu et al., 2002), agitation (Muñoz et al., 2004), sweeping gas (Muller-Feuga et al., 2012), circulation of cultures (Jiménez et al., 2003), airlift degassing column and $O_2$ absorption using perfluorocarbons (PFCs) (Wasanasathian and Peng, 2001), have been developed for use in microalgal cultures. However, their limitations cannot be ignored in practice. For instance, paddle wheel are applicable to open ponds only, mixer and stirrer may cause high shear stress to cells at high speed and are difficult to scale up; circulation of cultures and degassing column require large quantities of gas and consume much pumping energy; and the utilization of PFCs is of low efficiency and is not applicable to large scale. Under these circumstances, developing efficient and reliable de-oxygenation approach is demanded, particularly for large-scale microalgal farming. Hollow fiber membranes have been used as gas spargers in photobioreactors and have proven to be able to provide better gas distribution with low shear stress (Fan et al., 2007, 2008; Carvalho and Malcata, 2001). Nevertheless, it is not a deoxygenation mechanism and does not add to the efficiency of oxygen removal.

To this end, we demonstrated in this study with a 15-liter BCPBR equipped with a hollow-fiber membrane-based localized oxygen remover (LOR) (BCPBR-LOR) that localized oxygen removal is an effective deoxygenation mechanism, which could significantly mitigate oxygen accumulation in microalgal cultures. Enhanced lipid production of *N. oleoabundans* was also observed in BCPBR, hypothetically due to the lower dissolved oxygen ($dO_2$) in BCPBR-LOR as a result of the enhanced deoxygenation efficiency in comparison to BCPBR.

## 2. Gas exchange in BCPBR and BCPBR-LOR

As shown in Fig. 1a, in a BCPBR-LOR, air enriched with $CO_2$ is introduced from the sparger at the bottom, the same way as in a conventional BCPBR (Fig. 1b). However, the off gas, which is rich in oxygen, is collected by the hydrophobic hollow fibers and released from the top through the opening of these membranes. In comparison, the off gas is released from the top of the BCPBR. While BCPBR accumulates $O_2$ in the solar tube until the end of the tube, the BCPBR-LOR (Fig. 1c) separates the $O_2$ rich off gas locally along the entire length of the tube. The membrane also provides a large interface for over saturated oxygen in the liquid, which originates as a by-product of cellular photosynthesis, to escape into the membranes directly after travelling a relative short path.

## 3. Methods

### 3.1. Bubble column photobioreactor (BCPBR)

The 15-liter BCPBR is a custom-designed system, which was comprised of an acrylic tube (Canus Plastics Inc. Ottawa, Canada), a peristaltic pump for feeding (Masterflex L/S digital economy drive 77200-50, Cole-Parmer, Canada), $CO_2$ compressed gas cylinder and compressed air tubes. The schematic drawing and photos of the system are shown in Fig. 1a. The tube, which served as the vessel for housing microalgal culture and provided the surface for light harvesting, is 0.01 m in internal diameter (ID) and 1.83 m in height. A pH probe, a $dO_2$ probe, and a thermal couple were installed at the top of the tube for monitoring pH, $dO_2$, and temperature, respectively. A control panel of a Celligen Plus Cell culture system (Serial NO. 990525051, New Brunswick Scientific Co. Inc. Edison, New Jersey, USA) connecting all the probes were used for data acquisition via a computer. Air supply was mixed with a pure $CO_2$ stream (supplied by a $CO_2$ cylinder) to obtain 5% $CO_2$-enriched air, before being introduced into the tube. The flowrates of the air and $CO_2$ streams were controlled by flow meters ($CO_2$: 0–0.5 LPM scale, air: 0–5 LPM scale, Cole Parmer Canada Company, Montreal, Canada). The off-gas passed through a $CO_2/O_2$ gas analyzer (EX-2000 off-gas $CO_2/O_2$ ANALYZER, New Brunswick Scientific Edison, New Jersey, USA), which was connected to a computer for data acquisition, before being released into the ambience.

The same BCPBR was used as a BCPBR-LOR through minor modifications on the structure. As a BCPBR, $CO_2$-enriched air was sparged into the tube through the sparger at the bottom of tube, which was composed of six air sparging needles. Air bubbles rose through the culture to the freeboard of the tube at the top. As a BCPBR-LOR, a LOR was installed in the center of the tube. A LOR was composed of 12 hydrophobic hollow fiber membranes, which were fixed on a stainless steel hollow pillar by gluing at the bottom end and top end. The membranes were sealed at the bottom end but kept open at the top end, to allow collection of off gas along the fiber length since only gas could pass the hydrophobic membrane wall. Properties of the hollow membranes are listed in Table 1. Hydrophobic microporous hollow fibers (PURESEA SPRING Co., Ltd. Tianjin, China) were used as LOR to separate gas and liquid in this study.

### 3.2. Microalgal strain and medium

*N. oleoabundans* UTEX 1185 used in this study was purchased from the culture collection at the University of Austin (Texas). The modified Bristol medium (MBM), composed of (per liter, all analytical-grade) 0.35 g $NaNO_3$, 0.138 g $K_2HPO_4$, 0.0823 g $MgSO_4$, 0.025 g $CaCl_2$, 0.322 g $KH_2PO_4$, 0.025 g NaCl, 0.0068 g $FeCl_3$ and 1 mL of $A_5$ solution, was used as the medium for inoculation and cultivation. The $A_5$ solution was comprised of (per liter): 1.6423 g EDTA-Fe, 2.86 g $H_3BO_3$, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.22 g $ZnSO_4{\cdot}7H_2O$, 0.079 g $CuSO_4{\cdot}5H_2O$, 0.039 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. Besides, 160 mM $NaHCO_3$ was added in the media to ensure the non-sterile